mobility of the COMP digested fragment. In addition, EDTA chelator totally abolished the enzymatic activity. Take together; Zinc is essential for the ADAMTS7-mediated COMP degradation.
Cleavage of COMP by ADAMTS-7 is pH-dependent – ADAMTS-7 generated the largest amount of COMP fragments in the range of physiological pH (pH 7.5) up to pH 9.5, whereas the enzyme did not produce visible COMP fragments when pH values lower than 6.5 or higher than 10.5, indicating that the digestive activity of ADAMTS-7 is also pH-dependent.
Increased expression of ADAMTS-7 in the cartilage and synovium of patients with rheumatoid arthritis – To determine whether the expression of ADAMTS-7 in cartilage and synovium is altered in OA and/or RA, a real-time PCR was performed. ADAMTS-7 mRNA was significantly upregulated in RA cartilage, and only slightly upregulated in OA cartilage, compared to the normal control. Further analysis of synovium samples revealed that the level of ADAMTS-7 was also significantly upregulated in RA synovium compared to normal synovium.
Conclusion: ADAMTS-7 digests COMP in a Zn^{2+}- and pH-dependent manner. ADAMTS-7 is significantly upregulated in cartilage and synovium obtained from patients with RA, suggesting that ADAMTS-7 plays an important role in joint degenerative disease progression.
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THE INHIBITION OF CARTILAGE DEGRADATION MARKERS EXCRETION AFTER TREATMENT WITH RALOXIFENE IN WOMEN WITH KNEE OSTEOARTHRITIS

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Most drugs inhibiting bone resorption used in the treatment of osteoporosis decrease excretion of cartilage degradation markers and some of them stimulate chondrocytes and showed a protective effect on experimental arthropathies in animals. Among them, estrogens and raloxifene (RLX), SERM group, not only inhibit bone resorption but also act receptively on chondrocytes. Estrogens and HRT retarded progress of knee osteoarthritis but there is no evidence concerning a similar effect of RLX. In our parallely published work, there were statistically significant improvement of mean of WOMAC index which corelated with decrease of CTX-II excretion.
The aim of the study was to evaluate the effect of the 12-month-therapy with RLX in middle-aged women with knee osteoarthritis (OAK) and osteopenia on cartilage degeneration and bone turnovers markers.
The study was conducted in 65 women, aged 52-79, mean 65.4 years, with diagnosed OAK according to ACR criteria and rated on a 3-point Kellgren - Lawrence scale, with low bone mass (BMD of Hip neck and/or L1-L4 vertebrae T-score between –1.0 and –2.5), without contraindications to be treated with RLX. Patients were randomly assigned in the approximate ratio 1:2 to the control (C), or to group (E) being treated for 12 mounts with 60 mg of RLX p.o. per day. All the participants took Calcium up to 1200 mg/d plus 800 IU Vit. D_3 and Tramadol 1-2 x 50mg/24 hours in the case of pain. The evaluation of RLX effect on joint cartilage was based on measurements of excreted with urine the cartilage-specific C-terminal tetrapeptide of type II collagen CTX-II/Cr and cartilage oligometric matrix protein (COMP) with ELISA method. The bone turnover was monitored with bone-specific alkaline phosphatase (b-ALP), osteoprotegerin (OP), bone-specific C-terminal tetrapeptide of type I collagen (CTX-I), and soluble NF-kappa-B receptor activator (sRANKL) with ELISA method. The patients and markers were monitored before and after 3, 6, and 12 months of treatment with RLX.
Results: Regressive analysis of time changes showed statistically significant decrease in OP, CTX-II, CTX-II/Cr in the group treated with RLX and lack of any marker changes in the control group. The drop of excreted CTX-II and CTX-II/Cr after 12 months in group E was 30% and 40%, respectively with regard to the values before the treatment. There were neither differences in time and between the groups E and C as for markers of bone metabolism nor their correlation with markers of cartilage degeneration nor COMP changes.
Conclusion: We observed a statistically significant decrease in CTX-II and CTX-II/Cr excretion (with simultaneous significant lowering of WOAC index) in patients treated with RLX as compared to lack of such changes in the control group. It seems that RLX has a curative effect on cartilage degradation with improvement of clinical outcomes. There was no evidence on interaction between cartilage and bone metabolismexcept of decrease of OP.

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FRAGMENTS OF THE C-TELOPEPTIDE OF TYPE II COLLAGEN CAN BE DETECTED IN CIRCULATION CONCOMITANTLY WITH CLINICAL EVIDENCE OF JOINT DISEASE IN THE COLLAGEN INDUCED ARTHRITIS (CIA) MODEL OF RA IN THE RAT

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Background: Collagen induced arthritis in the rat has been extensively studied as a model of rheumatoid arthritis (RA). Disease activity and structural joint damage can be quantitatively assessed, and in addition biochemical markers have been adopted in the monitoring of disease progression in the CIA model. Helical fragments of type II collagen (C2C), which are generated by the action of aggrecanase, are slightly increased in the CIA model (Mayer et al, JBMR 2004;19:SA030). However, other proteases participating in the degradation of articular cartilage give rise to other collagen fragments, and therefore the present study was undertaken to investigate if fragments of C-telopeptide of type II collagen (CTX II) in circulation could reflect disease activity in this animal model of RA.
Objective: To investigate the time-course of collagen type II degradation in circulation in the CIA model of rheumatoid arthrtitis.
Methods: Twenty, 6-week old female Lewis (LEW/SsN/CrCrlBR) rats were randomized into 2 groups: (I) CIA group (15 animals) and (II) negative control (5 animals). The immunization was performed once at Day 1 with porcine type II collagen. Blood samples were collected at day 1 (prior to immunization), 8, 15, 18 and 23, and in addition, lavage synoviocentesis was performed at study termination. Cartilage degradation was evaluated using serum CTX-II (Nordic Bioscience, Denmark) and C2C (Ibex, Canada) assays. Bone resorption was estimated by CTX-I (Nordic Bioscience, Denmark). Paw inflammation was assessed semi-quantitatively using scores from 0 to 5.
Results: CIA group developed arthritis at Day 15 after immunization. The cumulative macroscopic scores from tarsal and carpal joints (left and right) were 0.0 and 9.1 $\pm$ 1.6 ($p = 0.0012$) in negative and CIA groups, respectively. Serum CTX II levels were increased 295% compared to controls at Day 15 ($p = 0.0012$). C2C was increased 12% in the CIA group, which, however, did not reach statistical significance. The cumulative macroscopic scores

at day 15 correlated with serum CTX-II (r = 0.72, p = 0.0004), but not with C2C (p = 0.132). Synovial CTX II measured at termination day was 251.9 ± 118.3 pg/ml (mean ± SD) and 7.64 ± 1.65 pg/ml in CIA and control groups, respectively. Finally, serum CTX-I did not shown any differences between groups until day 23. At Day 23, a 66% elevation in bone resorption was observed in the CIA animals compared to control group ($p < 0.05$).
Conclusion: C-telopeptide, but not helical, fragments of type II collagen is dramatically elevated in serum and synovial fluid as early as two weeks after immunization with type II collagen in the rat CIA-model. This accelerated cartilage degradation occurs simultaneously with disease onset as evaluated by paw inflammation. These observations suggest, that proteases different from aggrecanase are expressed early in this model of RA.

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RELEASE OF TYPE II COLLAGEN DEGRADATION FRAGMENTS HELIX-II AND CTX-II FROM ARTICULAR CARTILAGE BY CATHEPSINS AND MATRIX METALLOPROTEASES

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Aim: Type II collagen is abundantly and specifically found in cartilage and its degradation generates fragments such as HELIX-II and CTX-II. These fragments, which originate from the triple helical and C-telopeptide regions respectively, can be quantified using specific immunoassays (Syncart™ and Cartilaps™). Urinary HELIX-II and CTX-II concentrations are elevated in conditions of cartilage degradation, although their respective levels differ in OA and RA patients suggesting distinct collagenolytic pathways. The enzymes capable of cleaving type II collagen to release these fragments are currently unknown. We investigated the ability of cathepsins (Cat) and matrix metalloproteases (MMP) to digest native articular cartilage and to release the CTX-II and HELIX-II biomarkers.
Methods: Human hip articular cartilage (5 mg/mL) was digested with 1 μM human Cat B, L, K, S and D (pH 5.5) and 1 μM human MMP-1, -3, -7, -9 and -13 (pH 7.5) for 24 h at 37°C. The digestion of cartilage to release soluble peptides was determined by hydroxyproline and HELIX-II and CTX-II were determined by specific ELISAs.
Results: All cathepsins and MMPs solubilised between 10% and 70% of the native cartilage over 24 h, although the release of CTX-II and HELIX-II differed dramatically between enzymes. Cat B was the sole cathepsin capable of releasing significant quantities of CTX-II, whereas HELIX-II was only released efficiently by Cat L and S and to a lower extent by Cat K. CTX-II was released by all MMPs tested, although the efficiency differed for each enzyme. HELIX-II was released only by MMP-7, and to a lower extent by MMP-3. The addition of MMPs to collagen fragments released by Cat K from cartilage in a primary digestion, only released small quantities of CTX-II and no further HELIX-II. CTX-II produced by cartilage digestion with MMP-7 was not destroyed by subequent incubations with cathepsins whereas HELIX-II fragments were completely cleaved by subsequent incubation with Cat B.
Conclusion: Cathepsins and MMPs can both digest native cartilage *in vitro*, but only distinct subsets of these enzymes generate CTX-II and HELIX-II fragments. These findings may provide a biological rationale for the recent observation that the relative urinary abundance of HELIX-II and CTX-II differs between RA and OA patients and support the concept that specific protease inhibitors could be used selectively against given arthritis diseases.

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QUANTITATION AND RELATIVE CHAIN LENGTH DETERMINATION OF HYALURONAN (HA) AND CHONDROITIN SULFATE (CS) IN SERUM AND SYNOVIAL FLUID

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Aim of study: to quantitate and determine relative chain lengths of HA and CS fragments in serum and synovial fluid.
Methods: After proteinase K digestion, serum (1 ml) and synovial fluid (0.25 ml) samples were fractionated by gel filtration columns packed with allyl dextran/bisacrylamide (Sephacryl S-1000) or agarose (Superose 6). S-1000 has a fractionation range (M_r) of 5×10^5 to $>10^8$ and Superose 6 has a range up to 10^6. Larger fragments were eluted first, followed by progressively smaller fragments. The columns had values of $V_0 \sim 6$ ml and $V_t \sim 25$ ml. Fractions (1 ml) 7 through 24 were collected and analyzed (S-1000 fractions designated A7 - A24 and Superose 6 fractions designated B7 - B24). For serum, only Superose 6 fractions were analyzed. Fractions were digested with chondroitinase ACII/ABC and the disaccharide digests were labeled with 2-aminoacridone for fluorophore-assisted carbohydrate electrophoresis (FACE). FACE gels were photographed and analyzed with Kodak 1D software.
Results: Fluorotagged HA and CS digestion products from

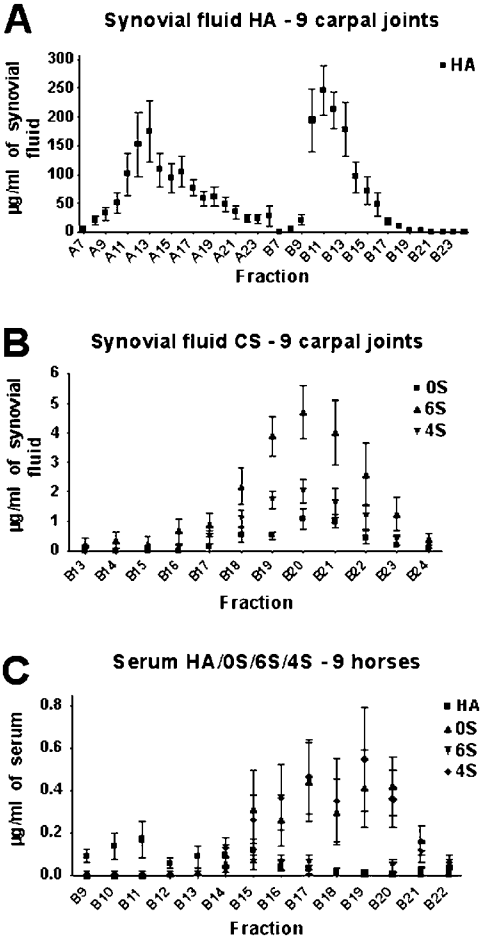

Fig. 1. HA/0S/6S/4S content in S-1000 fractions (A7-A24) and Superose 6 fractions (B7-B24) of synovial fluid and serum from 9 normal horses. A: Synovial fluid HA content. B: Synovial fluid CS content. C: Serum HA/0S/6S/4S content.